K092736

ATTACHMENT 1

PREMARKET NOTIFICATION
510(K)
SAFETY AND EFFECTIVENESS SUMMARY
(as required by 21 CFR § 807.92)

SEP 2 2010

A. 510(k)Number:
K092736

B. Purpose for Submission:
New device

C. Measurand:
Anti-M2-3E autoantibodies

D. Type of Test:
Qualitative or semi-quantitative enzyme immunoassay

E. Applicant:
EUROIMMUN US INC.

F. Proprietary and Established Names:
EUROIMMUN Anti-M2-3E ELISA (IgG)

G. Regulatory Information:

1. Regulation:
   21 CFR 866. 5090- Antimitochondrial antibody immunological test system
2. Classification:
   Class II
3. Product code:
   DBM
4. Panel:
   Immunology

H. Intended Use:

1. Intended use(s):
   The EUROIMMUN Anti-M2-3E ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against the mitochondrial antigens M2 in human serum and plasma. It is used as an aid in the diagnosis of primary biliary cirrhosis (PBC), in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
   Same as intended use.
3. Special conditions for the use statement(s):
   For prescription use only.
4. Special instrument requirements:
   Microwell plate reader capable of measuring OD at 450nm and at 620nm for dual wavelength readings.

I. Device Description:
The EUROIMMUN Anti-M2-3E ELISA (IgG) consists of a microwell ELISA plate coated with M2-3E antigen, 3 calibrators, positive and negative control, peroxidase-labelled anti-human IgG conjugate, sample buffer, wash buffer concentrate, TMB chromogen/substrate solution and stop solution.

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J. Substantial Equivalence Information:

1. Predicate device name (s):
   Inova Quanta Lite M2 EP (MIT3) ELISA
2. Predicate 510(k) number(s):
   K052262
3. Comparison with predicate:

Similarities		
Item	**New Device**	**Predicate Device**
Intended use	Detection of IgG antibodies to mitochondrial antigens as an aid in diagnosis of primary biliary cirrhosis (PBC) and overlap syndrome with autoimmune hepatitis.	Detection of IgG antibodies to mitochondrial antigens as an aid in diagnosis of primary biliary cirrhosis.
Technology	ELISA	Same
Assay platform	96-well microtiter plates	Same
Calibration	Relative units	Same
Conjugate	Rabbit anti-human IgG labeled with horseradish peroxidase	Goat anti-human IgG labeled with horseradish peroxidase
Substrate	TMB	Same
Reagent preparation	All reagents, calibrators and controls are ready to use, except for the wash buffer.	Same
Procedure	Sample incubation with micro-well antigen coated plate, followed by a wash step, incubation with an anti-human IgG enzyme conjugate; wash step, incubation with substrate; then the addition of a stop solution and reading at 450nm.	Same
Differences		
Item	**New Device**	**Predicate Device**
Assay format	Qualitative or semi-quantitative (using either the 3 calibrators or 1 calibrator only)	Semi-quantitative
Antigen	Mixture of pyruvate dehydrogenase (isolated from porcine heart) and a recombinant fusion protein. The recombinant protein was produced in E.coli and comprises the immunogenic domains of the E2 subunits from branched-chain 2-oxo-acid dehydrogenase (BCOADH), pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGDH), together called BPO.	Affinity purified recombinant M2 EP MIT3
Calibrators	3 calibrators 2, 20 and 200 RU/ml	
Controls	2 controls 1 positive, 1 negative	3 controls 1 high positive, 1 low positive, 1 negative
Stop solution	0.5 M sulphuric acid	
Samples	Serum or plasma 1:101 dilution	Serum 1:101 dilution
Reported units	RU/ml or Ratio	Units
Cut Off level	20 RU/ml	25 Units

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

Patient samples are diluted 1:101 in sample buffer, 100 µl of each diluted patient sample and pre-diluted controls and calibrators are added to the antigen coated microtiter wells and incubated for 30 minutes at room temperature. After incubation the microtiter well strips are washed with wash buffer to remove unbound antibodies and 100 µl of the anti-human IgG enzyme conjugate reagent is added to each microtiter well. After an additional 30-minutes incubation at room temperature, the microtiter wells are again washed 3 times with 300 µl of wash buffer to remove any unbound enzyme conjugate and 100 µl of the chromogen substrate is added. The strips are incubated for 15 minutes at room temperature and 100 µl stop solution is added. The microtiter plates are placed in an ELISA reader and read at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes.

M. Performance Characteristics (where applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days. Neither inter-assay variation nor intra-assay variation should show results over CV = 12% for positive samples. The following results were obtained:

Intra-assay reproducibility

n = 20	Anti-M2-3E ELISA (IgG) RU/ml							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean value (x):	144	101	70	30	101	10	45	19
Standard deviation (SD):	2.26	2.23	1.60	1.01	2.34	1.05	1.37	0.69
Coefficient of variation (CV, %):	1.6	2.2	2.3	3.4	2.3	10.5	3.1	3.7

n = 20	Anti-M2-3E ELISA (IgG) RU/ml			
	Sample 9	Sample 10	Sample 11	Sample 12
Mean value (x):	5.5	5.0	16.1	21.8
Standard deviation (SD):	0.34	0.29	0.68	1.21
Coefficient of variation (CV, %):	6.2	5.9	4.3	5.5

Mean CV (%):	4.3

Inter-assay reproducibility

n = 4 tests / 6 days = 24	Anti-M2-3E ELISA (IgG) RU/ml							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean value (x):	158	103	67	43	80	10	39	27
Standard deviation (SD):	4.69	6.20	5.77	4.42	6.30	0.85	2.52	1.95
Coefficient of variation (CV, %):	3.0	6.0	8.6	10.3	7.9	8.4	6.5	7.2
Mean CV (%):	7.2							

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n = 20	Anti-M2-3E ELISA (IgG) RU/ml			
	Sample 9	Sample 10	Sample 11	Sample 12
Mean value (x):	5.6	4.9	18.2	24.9
Standard deviation (SD):	0.66	0.39	1.91	2.31
Coefficient of variation (CV, %):	11.9	8.0	10.5	9.3
Mean CV (%):	8.1			

The Lot to Lot reproducibility is checked during the validaton of the kit. 3 different lots are incubated with different QC samples each. For each sample the coefficient of variation (CV) is calculated. Inter-lot variation should show results below CV = 12% for positive samples. The following results were obtained:

Lot to lot reproducibility

n = 3 lots x 2 runs = 6	Anti-M2-3E ELISA (IgG) RU/ml							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean value (x):	14	26	19	27	56	90	98	164
Standard deviation (SD):	1.33	1.33	1.38	1.86	2.51	5.01	7.73	8.96
Coefficient of variation (CV, %):	9.6	5.1	7.5	7.0	4.5	5.6	7.9	5.5
Mean CV (%):	6.6							

b. *Linearity/assay reportable range:*

The linearity of the test was investigated using serial dilutions of patient sera with high antibody concentrations. Each patient serum was diluted up to 1/32. The dilutions were measured with the Anti-M2-3E ELISA (IgG) according to the package insert in single determinations and the results calculated in RU/ml. The observed/expected (O/E) values in the area of the cut-off were within the specifications (0.8 > O/E > 1.2 - out of specification values are highlighted in grey).

Dilution	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5		
	Exp. RU/ml	Obs. RU/ml	O/E	Exp. RU/ml	Obs. RU/ml	O/E	Exp. RU/ml	Obs. RU/ml	O/E	Exp. RU/ml	Obs. RU/ml	O/E	Exp. RU/ml	Obs. RU/ml	O/E
1/1	199	199	1.0	161	161	1.0	159	159	1.0	140	140	1.0	198	198	1.0
1/2	99	98	1.0	80	110	1.4	79	99	1.2	70	73	1.0	99	103	1.0
1/4	49	57	1.2	55	61	1.1	49	53	1.1	36	41	1.1	51	61	1.2
1/8	28	26	0.9	31	28	0.9	27	21	0.8	21	17	0.8	30	30	1.0
1/16	13	12	0.9	14	11	0.8	10	9	0.9	9	8	0.9	15	12	0.8
1/32	6	6	0.9	6	5	0.8	4	3	0.7	4	3	0.8	6	5	0.8

c. *Traceability, Stability, Expected values (controls, calibrators or methods):*

There is no recognized standard or reference material for anti-mitochondrial antibodies. Arbitrary units are used in the assay.

d. *Detection limit:*

The limit of blank is the lowest arbitrary concentration from a replicate series of samples without analyte. It is defined for this assay as a value of three times the standard deviation of the sample buffer. The detection limit of the Anti-M2-3E ELISA (IgG) is 1.1 RU/ml, determined from 20 replicate determinations of sample buffer only. Due to the high dilution factor of the samples, no influence of the sample material (serum or plasma) is expected. The lowest quantified concentration is the lowest calibrator used in the assay (2 RU/ml).

e. *Analytical specificity:*

Cross-reactivity: The quality of the antigen used and the antigen source ensure a high specificity of the ELISA. Cross reactivity was investigated using a panel of 29 sera serologically positive for antibodies against PCA (n = 10), GBM (n = 10) and LKM (n = 9). All 29 sera were negative in the Anti-M2-3E ELISA (IgG), so no cross reactivity is expected.

Interference: To investigate the influence from hemoglobin, triglycerides and bilirubin, 5 different specimens at different anti-M2-3E concentrations (4 – 186 RU/ml) were spiked with potential interfering substances and were incubated with the test system. The recovery in relation to the unspiked sample without interferent was calculated. The individual recovery was within the range of 98 – 123 %. No significant interference was observed for concentrations of up to 1000 mg/dl for hemoglobin, 2000 mg/dl for triglyceride and 40 mg/dl for bilirubin.

f. *Assay cut-off:*

Qualitative evaluation: Ratio 1.0

Semi-quantitative evaluation: 20 RU/ml

2. Comparison studies:

a. *Method comparison with predicate device:*

An external clinical study was performed with 120 clinically characterized samples (39 from AIH patients, 49 from PBC patients and 32 from patients with AIH/PBC overlap syndrome) collected at EUROIMMUN UK Ltd. The samples were tested with the EUROIMMUN Anti-M2-3E ELISA (IgG) and with the Inova Quanta Lite M2 EP (MIT3) ELISA as the predicate device. The panel consisted of 18 men and 100 women (2 unknown). The age ranged from 1 - 87 years (2 unknown) with an average age of 50 years. To cover the range around the cut-off with more samples, additional 7 samples with concentrations near the cut-off were created by mixing of positive and negative samples and the results included in the comparison.The results are shown in the table below.

Of the 9 discrepant samples negative in the Inova test and positive in the EUROIMMUN assay, 2 were from patients with AIH/PBC overlap syndrome, 3 were from AIH-1 patients, one from a PBC patient and 3 from an antibody-positive patient diluted with negative material to fit the borderline range. The discrepant sample positive in the Inova test and negative in the EUROIMMUN assay also was a created sample.

n = 127		Inova Quanta Lite M2 EP (MIT3) ELISA		
		positive	borderline	negative
EUROIMMUN Anti-M2-3E ELISA (IgG)	positive	78	2	9
	negative	1	2	35

Positive agreement	78	/	79	=	98.7%	95% C.I.:	93.1%	-	100.0%
Negative agreement	35	/	44	=	79.5%	95% C.I.:	64.7%	-	90.2%
Overall agreement	113	/	123	=	91.9%	95% C.I.:	85.6%	-	96.0%

Results of samples within the measurement range (2-200 RU/ml) only (including mixed samples)

n = 46		Inova Quanta Lite M2 EP (MIT3) ELISA		
		positive	borderline	negative
EUROIMMUN Anti-M2-3E ELISA (IgG)	positive	24	2	9
	negative	1	1	9

Positive agreement	24	/	25	=	96.0%	95% C.I.:	79.6%	-	99.9%
Negative agreement	9	/	18	=	50.0%	95% C.I.:	26.0%	-	74.0%
Overall agreement	33	/	43	=	76.7%	95% C.I.:	61.4%	-	88.2%

b. *Matrix comparison:*
The usability of plasma was investigated using 21 sample pairs each of serum and corresponding plasma. The samples cover concentrations in the diagnostically important range, i.e. the lower area of the calibration curve and the cut-off. Passing-Bablok regression was calculated for the comparison of serum to plasma. The results are shown in the table below.

	EDTA plasma	Heparin plasma	Citrate plasma
Regression equation (y = plasma, x = serum)	y = 0.09 + 1.01 x	y = -0.10 + 0.98 x	y = 0.56 + 1.02 x
95% C.I. of intercept	-1.30 – 2.75	-1.32 – 1.11	-1.02 – 3.18
95% C.I. of slope	0.98 – 1.04	0.96 – 1.01	0.98 – 1.06

A comparison in which the 95% C.I. of the slope contains 1.0 and the 95% C.I. of the intercept contains 0 indicates equivalence of concentration between serum and the corresponding plasma matrices. The comparisons above satisfy this condition.
Coefficients of determination were found to be above 0.99 and %BIAS from serum was in the range of 82 to 112 % (serum = 100 %).

3. Clinical studies:

In a clinical study, performed in cooperation with several university hospitals (see below), in total 1180 clinically characterized samples (251 from PBC patients and 929 from control groups) were investigated for anti-mitochondrial antibodies (IgG). The EUROIMMUN Anti-M2-3E ELISA (IgG) showed a sensitivity for PBC of 92.8% and a specificity of 97.4%. The results are shown in the table below. 95% C.I. are calculated by the exact method.

a. *Sensitivity:*

No.	Panel	n (men, women)	Mean age (age range)	Anti-M2-3E ELISA (IgG) positive	%	95% C.I.
1	Primary biliary liver cirrhosis	251 (20, 229, 2 unknown)	55 y (22 – 85 y)	233	92.8%	88.9 – 95.7%

No.	Panel	n (men, women)	Mean age (age range)	Anti-M2-3E ELISA (IgG) positive	%	95% C.I.
2	PBC/AIH overlap syndrome	15 (1, 14)	55 y (32 – 87 y)	15	100.0%	78.2 – 100.0%

b. *Clinical specificity:*

No.	Panel	n (men, women)	Mean age (age range)	Anti-M2-3E ELISA (IgG) negative	%	95% C.I.
3	Autoimmune hepatitis	131 (20, 65, 46 unknown)	40 y (1 – 86 y, 46 unknown)	125	95.4%	90.3 – 98.3%
4	Viral hepatitis	239 (16, 23, 200 unknown)	48 y (25 – 84 y; 200 unknown)	239	100.0%	98.5 – 100.0%
5	Primary sclerosing cholangitis	19 (12, 7)	48 y (21 – 73 y)	19	100.0%	82.4 – 100.0%
6	Systemic lupus erythematosus	100 (9, 91)	42 y (18 – 79 y)	92	92.0%	84.8 – 96.5%
7	Sjogren's syndrome	120 (8, 112)	52 y (18 – 81)	112	93.3%	87.3 – 97.1%
8	Rheumatoid arthritis	120 (33, 87)	54 y (23 – 80)	118	98.3%	94.1 – 99.8%
9	Asymptomatic blood donors	200 (131, 69)	40 y (19 – 69 y)	200	100.0%	98.2 – 100.0%
	Total	929		905	97.4%	96.2 – 98.3%

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off:
See Assay cut-off.

5. Expected values/Reference range:
The levels of anti-mitochondrial antibodies (IgG) were analyzed with the EUROIMMUN Anti-M2-3E ELISA (IgG) in a panel of 200 apparently healthy blood donors, consisting of 120 men and 80 women with an age range of 19-69 years (average age: 40 years). With a cut-off of 20 RU/ml, all blood donors were found negative.

Positives	0
Negatives	200
Lowest value	0.35 RU/ml
Highest value	14.50 RU/ml
Mean value	3.81 RU/ml
Std dev. (SD)	2.944 RU/ml

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

Date

Signature

Kathryn Kohl, Managing Director
Typed Name, Title

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food & Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993

EUROIMMUN US INC.
c/o Ms. Kathryn Kohl
Managing Director
429 Rockaway Valley Road
Unit 1200
Boonton Township, NJ 07005

SEP 0 2 2010

Re: k092736
Trade/Device Name: EUROIMMUN Anti-M2-3E ELISA (IgG)
Regulation Number: 21 CFR§866.5090
Regulation Name: Antimitochondrial Antibody Immunological Test System
Regulatory Class: Class II
Product Code: DBM
Dated: July 22, 2010
Received: July 30, 2010

Dear Ms. Kohl:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must

comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M Chan

Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

ATTACHMENT 2

INDICATIONS FOR USE STATEMENT

K092736

SEP 0 2 2010

510(k) Number (if known): K092736

Device Name: Anti-M2-3E ELISA (IgG)

Indications For Use:

The EUROIMMUN Anti-M2-3E ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against the mitochondrial antigens M2 in human serum and plasma. It is used as an aid in the diagnosis of primary biliary cirrhosis (PBC), in conjunction with other laboratory and clinical findings.

Prescription Use __X__ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (OIVD)

Reena Philip

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510K k092736

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